Immobilization of Simulated Reducing Agent at the Surface of SiO₂ Fillers in Dental Composite Resins

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To reduce the leachability of reducing agents from composite resins, immobilization of a simulated reducing agent at the surface of SiO₂ fillers was examined. SiO₂ plates were immersed in 2% 3-aminopropyltriethoxy silane/ethanol solution, and then immersed in dimethyl sulfoxide with 0.25 wt% 4-dimethyl amino benzoic acid (DMABA), 2.0 wt% 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, and 0.5 wt% *N*-hydroxysuccinimide. Wide-scan spectrum of X-ray photoelectron spectroscopy did not detect carbon contamination. However, narrow scan detected an O=C–N peak at 399.8 eV, suggesting that DMABA could be immobilized on silane-coupled SiO₂ plates. Further, surface plasmon resonance analysis indicated the adsorption of MMA at the surface of reducing agent-immobilized plate.

Keywords: Immobilization, Simulated reducing agent, SiO₂ fillers

INTRODUCTION

Recently, the concept of minimal intervention (MI) has been a focus in the dental field^{1–5}, especially for conservative dentistry. From the viewpoint of minimal intervention dentistry, composite resins are the preferred material of choice. This is because apart from their advantages of high esthetic property and reliable durability, composite resins and glass ionomer cements applied in minimal intervention can provide high-quality preventive and restorative care to a population at high risk of caries⁶.

Composite resins are composed of a hydrophobic matrix resin and hydrophilic fillers — which when combined render the composite resin with better properties than each base material. In addition, they contain reducing agent, polymerization initiator, and polymerization inhibitor — which when taken together, contribute to the degree of polymerization, stability, and handling properties.

In particular, light-activated composite resins afford a number of advantages over chemically activated resins. Light-activated composite resins are single-component pastes that require no mixing. The free radical initiating system, consisting of the photoinitiator molecule and an amine activator, is contained in this paste. On this note, a number of amine accelerators are suitable for interaction with camphorquinone, such as dimethylaminoethyl methacrylate (DMAEMA) or 4-dimethyl amino benzoic acid ethylester (DMABEE).

However, some residual monomers and reducing agents leach from the surface of polymerized

composites and are doubted as potential endocrine disrupting chemicals (EDCs), so-called “environmental hormones”⁷. The work of Olea *et al.*⁸ on the detection of bisphenol A in a Bis-GMA-based sealant led to the recognition of potential problems posed by EDCs in clinical dentistry. EDCs interfere with normal male reproductive development by acting as environmental estrogens or anti-androgens, or *via* other potential mechanisms⁹. On the other hand, Jager and Rubin¹⁰ reported on the presence of a plasticizer, di(2-ethylhexyl) phthalate (DEHP), in the tissues and organs of two deceased patients who had previously received blood transfusions. The blood had been stored in bags made of polyvinyl chloride (PVC) plasticized with DEHP. In view of the growing concern that phthalate esters may be a threat to health and to our ecological system, there is an imperative and urgent need to further investigate and review the toxicity and possible health threats that these esters may present directly or indirectly to man¹¹.

In the dental field, soft resins are widely used for short-term temporary restorations after cavity preparation and as denture materials in dentistry. Their flexibility is due to the presence of plasticizer additives such as phthalate esters. Although phthalate esters are often used because of their very low toxicity, there is growing concern that they may cause endocrine disruption. In fact, recent environmental toxicology research has found — *in vitro* and *in vivo* — that some phthalate esters are EDCs^{12–15}. Phthalate esters are an ortho form of benzenedicarboxylic acid prepared by a reaction of

phthalic acid with a specific alcohol to form the desired ester. As for chemically similar benzoic ester, 4-dimethyl amino benzoic acid ethylester (DMABEE) a reducing agent, it is also considered an environmental hormone in the dental field. It has been reported to exhibit cell toxicity, inducing death by apoptosis¹⁶⁻¹⁹.

In this study, we examined the immobilization of 4-dimethylamino benzoic acid (DMABA) as a simulated reducing agent for DMABEE at the surface of SiO₂ fillers to protect against leachability and to improve composite properties. DMABA can be reduced and has an immobilizing possibility, while DMABEE cannot be immobilized.

MATERIALS AND METHODS

Immobilization of reducing agent on SiO₂ plate

For surface decontamination, SiO₂ plates (Lot. No. SK-4303, Sumikin Sekiei Co. Ltd., Tokyo, Japan) were boiled in 0.5 wt% sodium peroxodisulfate aqueous solution (Katayama Co. Ltd., Osaka, Japan) for 15 minutes and rinsed ultrasonically with acetone for 30 minutes²⁰⁻²².

For silanization, these plates were immersed in 2% 3-aminopropyltriethoxy silane (APTES, Chisso Co. Ltd., Tokyo, Japan)/ethanol solution at room temperature for 30 minutes and rinsed ultrasonically

with ultrapure water (Milli-Q water) for five minutes.

In this study, 4-dimethyl amino benzoic acid (DMABA; Tokyo Chemical Industry, Tokyo, Japan) was used as the simulated reducing agent, because it has a structure similar to 4-dimethyl amino benzoic acid ethylester (DMABEE) which is used as a reducing agent in the dental field. Silanized plates were immersed in dimethyl sulfoxide (DMSO; Sigma - Aldrich Japan Co. Ltd., Tokyo, Japan) with 0.25 wt% DMABA, 2.0 wt% 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC; Dojindo Co. Ltd., Kumamoto, Japan), and 0.5 wt% *N*-hydroxysuccinimide (NHS; Wako Pure Chemical Ind. Ltd., Osaka, Japan) for one hour, and rinsed ultrasonically with acetone for five minutes. The chemical structures of APTES, DMABEE, DMABA, EDC, and NHS are shown in Fig. 1. SiO₂ plates appended with simulated reducing agent by a chemical reaction using EDC and NHS are indicated in Fig. 2.

Preparation of SiO₂ surface model on gold-coated glass plate

Glass plates made of BK-7 (refractive index: 1.515, size: 15 mm in diameter, 1.0 mm in thickness; Matsunami Glass Ind. Ltd., Osaka, Japan) were coated with gold using an ion coater (Osaka Vacuum

Fig. 1 Chemical structures of 3-aminopropyltriethoxy silane (APTES), 4-dimethyl amino benzoic acid ethylester (DMABEE), 4-dimethyl amino benzoic acid (DMABA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and *N*-hydroxysuccinimide (NHS).

Fig. 2 Immobilization of 4-dimethyl amino benzoic acid (DMABA) on SiO₂ plates with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS).

Industrial Co. Ltd., Osaka, Japan). The gold-coated glass plates were prepared by depositing Cr (1 nm) first, and then Au (49 nm) immediately under 2.0×10^{-2} Pa. The metal target was heated by an electron beam.

Gold-coated glass plates were immersed in a piranha solution (7:3 mixture of concentrated sulfuric acid and 30% hydrogen peroxide solution) for five minutes at room temperature. After rinsing three times with distilled water, they were stored in 2-propanol until use.

11-mercapto-1-undecanol (Sigma - Aldrich Japan Co. Ltd., Tokyo, Japan), which has a terminal hydroxyl group, was dissolved in anaerobic ethanol to 1 mM under bubbling nitrogen gas. Gold-coated glass plates were immersed in the alkanethiol solution for 24 hours to prepare a self-assembled monolayer (OH-SAM) on the gold surface and sequentially washed with distilled water and 2-propanol. These plates, as the SiO₂ surface model, were silanized and the reducing agent immobilized as follows. The gold-coated glass plates immobilized with simulated reducing agent were immersed in methyl methacrylate (MMA) with 0.4% camphorquinone and irradiated with light for five minutes using -LIGHT II (Morita Co. Ltd., Saitama, Japan). They were sequentially washed with heated acetone for three hours by Soxhlet extraction to polymerize, and then the immobilized PMMA layer was washed. Gold-coated glass plates with OH-SAM were processed similarly and used as a control.

X-ray photoelectron spectroscopy (XPS)

The surfaces of SiO₂ with APTES and DMABA were chemically analyzed by XPS (AXIS-HS, Kratos, Manchester, UK) with an AlK α monochromatic X-ray source. Wide- and narrow-scan spectra were acquired at pass energies of 80 and 40 eV, respectively. The chemical states of N 1s were identified from detailed measurement of peak positions.

Surface plasmon resonance (SPR)

Adsorption amounts of OH-SAM, APTES, DMABA, and PMMA on the gold-coated glass plates were estimated using SPR spectra^{23,24}. After measuring the SPR spectra of each processing stage, each adsorption amount was assessed from the SPR angle shift using Fresnel's law for the multilayer system, BK-7/Cr/Au/OH-SAM/APTES/DMABA/PMMA/Air.

RESULTS

Analysis of XPS wide- and narrow-scan spectra

XPS detected Si and O as basic constituents at the surface of decontaminated SiO₂ plates washed by sodium peroxodisulfate, but did not detect carbon contamination. However, at the surface of silane-coupled SiO₂ plate and reducing agent-immobilized SiO₂ plate, XPS detected C and N, which were not detected on decontaminated SiO₂ plate. Silane-coupled SiO₂ plate did not show a big difference when compared with reducing agent-immobilized SiO₂ plate (Fig. 3a). SiO₂ fillers showed the same results as

Fig. 3 XPS wide-scan spectra of: (a) SiO₂ plate and (b) SiO₂ filler.

Fig. 4 XPS narrow-scan spectra of SiO₂ plates (a, b) and SiO₂ fillers (c, d). a, c: silane treatment; b, d: with simulated reducing agent.

Fig. 5 SPR angle of each treated sample: (a) control; (b) OH-SAM; (c) silane-coupled OH-SAM; and (d) silane-coupled OH-SAM immobilized with simulated reducing agent.

Fig. 6 Observation of the shifts of SPR angles showing the adsorption of MMA at the surface of: (a) control; (b) OH-SAM; (c) silane-coupled OH-SAM; and (d) silane-coupled OH-SAM immobilized with simulated reducing agent.

SiO₂ plates (Fig. 3b).

However, in N 1s narrow-scan spectra by XPS, a difference between silane-coupled SiO₂ plate and reducing agent-immobilized SiO₂ plate was observed. XPS detected an N - H peak at 398.8 eV of binding energy at the surface of silane-coupled SiO₂ plate. On the other hand, XPS detected an O = C - N peak at 399.8 eV of binding energy at the surface of reducing agent-immobilized SiO₂ plate which was not detected at the surface of silane-coupled SiO₂ plate, as well as N - H peak at 399.3 eV of binding energy (Figs. 4a and 4b). SiO₂ fillers showed the same results as SiO₂ plates (Figs. 4c and 4d).

Analysis of SPR

Between the control and OH-SAM plate, there was a change of 101.6 mDA of the SPR angle. In addition, a 25.4 mDA shift of the SPR angle was observed between OH-SAM plate and silane-coupled OH-SAM

plate, and a shift of 13.6 mDA between silane-coupled OH-SAM plate and reducing agent-immobilized, silane-coupled OH-SAM plate. Therefore, 50.8 ng/cm² was adsorbed after OH-SAM, 12.8 ng/cm² after silane coupling, and 6.8 ng/cm² after immobilization of reducing agent (Fig. 5).

Moreover, in OH-SAM plate, the SPR angles before and after light irradiation in MMA were 43524.7 mDA and 43556.2 mDA respectively. There was no apparent difference between pre- and after post-irradiation in MMA. Little adsorption of MMA was observed at the surface of OH-SAM plate. In reducing agent-immobilized plate, however, the SPR angles before and after light irradiation in MMA were 43797.9 mDA and 44328.5 mDA respectively. The SPR angle shifted by 530.6 mDA between before and after light irradiation in MMA. Therefore, MMA adsorption was apparent at the surface of reducing agent-immobilized plate (Fig. 6).

DISCUSSION

3-aminopropyltriethoxy silane (APTES), which has a structure similar to -methacryloxy propyl trimethoxy silane (-MPTS), is deemed as the best silane coupling agent of dental composite resins. This is chiefly because APTES has three different functional groups for reaction with the filler.

In this study, the silane coupling layer was composed of three layers: outer layer, middle layer, and base layer^{25,26}. From the XPS results, it was apparent that the silane coupling agent and reducing agent were adsorbed at the surface of SiO₂ plates and fillers after each process of silanization and immobilization of simulated reducing agent. However, in the present study, hardly any difference was observed between the SiO₂ plates and fillers in the peaks of XPS wide-scan spectra. This was probably due to the lack or absence of the covering effect of the outer and middle layers. The outer and middle layers were easily destroyed by water and warm water respectively, because they were formed by the mutual reaction of the respective agents. However, the base layer strongly bound with the surfaces of the plate and filler by siloxane bridge formation.

It was possible that the outer and middle layers were removed by subsequent washing, even if the carboxyl group of the simulated reducing agent was bound chemically by EDC and NHS with the amino group in the outer and middle layers. Nonetheless, to measure more accurately the reaction between the amino group of silane coupling agent and the carboxyl group of simulated reducing agent by EDC and NHS, and to set the amide bond on the surface of plates and fillers, the outer and middle layers of the silane coupling agent were removed.

In the N 1s narrow-scan spectra of XPS, N - H and O=C - N peaks were observed after immobilization of the simulated reducing agent, while only N - H was observed after silanization. The immobilization method of the simulated reducing agent using EDC and NHS could bind the amino group with the carboxyl group as an amide bond. This method was originally used to immobilize proteins to collagen. Following immobilization of simulated reducing agent, ultrasonic cleaning was performed for five minutes in acetone before acquiring the XPS spectra. Nevertheless, the peak of O=C - N was observed. It was thought that the simulated reducing agent was immobilized strongly by the chemical bond at the surface of the silane coupling agent. As a result, the peak of O=C - N of the amide bond appeared after immobilization of the simulated reducing agent.

In the SPR results, the incident angle shifted by about 530 mDA before and after light irradiation in MMA. The amount of MMA adsorbed at the surface of the plate was approximately 265 ng/cm² for the gold-coated plate immobilized with simulated reducing agent. It seemed unlikely to consider that PMMA chains were bound at the surface of the silane coupling agent, because APTES has a terminal amino group but not a functional group which binds with matrix resins like the methacryloxy group. However, the adsorption of MMA was clearly observed at the surface of the gold-coated plate although it was washed with heated acetone for three hours by Soxhlet extraction before SPR measurement.

MMA is an organic solvent that dissolves easily in acetone. It was thought that the PMMA chain polymerized by light irradiation bound to the surface of the plate by strong binding like a chemical bond given that it did not dissolve in acetone even at high temperature. In this research, it was suggested that PMMA chains bound to the surface of the simulated reducing agent or the silane coupling agent by chemical bonds, although the chemical structure could not be clarified.

EDC and NHS, as crosslinking agents employed for the present immobilization method, are safe to handle, comparatively cheap, and that they facilitated a short immobilization time. APTES, as a silane coupling agent, contains an active terminal amino group. Findings of this study indicated that these three agents were useful for the safe and comparatively easy immobilization of 4-dimethyl amino benzoic acid a simulated reducing agent with a carboxyl group at the end at the surface of SiO₂ plates and fillers. Moreover, it was suggested that the simulated reducing agent could be immobilized by optical means at the surface of composite resins.

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